amended page 36 of the specification to reflect the new address of the American Tissue Type Culture Collection.

Applicants have canceled pending claims 16, 17, 20, 23-42, 44, 46, 47, 49 and 50 and added claims 51-72, as discussed more fully below in response to the outstanding Office Action. Applicants believe that presentation of the claims in a contiguous format will facilitate the Examiner's continued review of this application. Applicants' cancellation of claims, which is not to be interpreted as acquiescence to the outstanding rejections, is without prejudice or waiver of their right to pursue the subject matter of those claims in a co-pending application or in future applications claiming priority herefrom under 35 U.S.C. § 120. For the Examiner's convenience, applicants have enclosed, as Exhibit A, a table correlating the added and former claims. None of the amendments or added claims constitutes new matter. Applicants address below the outstanding rejections.

The Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 16-17, 20, 23, 24, 26-37, 40-42, 44, 46, 47, 49 and 50 stand rejected under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention". More particularly, with respect to claims 16-17, 20, 23, 24, 26-30, 34-36, 44, 46 and 49, the Examiner

19contends the specification does not define the term "specifically hybridize". This rejection is obviated by the cancellation of those claims.

None of added claims 51-72 recites the term "specifically hybridize". Instead, the added claims utilize the term "hybridize". Hybridization-based claims constitute established claim terminology in the recombinant DNA field. Such terminology, in the absence of specific hybridization conditions, has been accepted by the United States Patent and Trademark Office. See, for example, the United States patents listed below (copies enclosed as Exhibit B), which contain claims to DNA sequences that hybridize to a specifically disclosed DNA sequence:

1. 4,960,700 (Genentech) - mammalian enkephalinase (claim 1);
2. 4,978,621 (Scripps Clinic) - merozoite surface antigens (claims 1 and 2);
3. 5,030,722 (Johns Hopkins) - rat odorant binding protein (claim 1);
4. 5,037,756 (US Army) - human terminal transferase (claim 1);
5. 5,075,222 (Synergen) - interleukin 1 inhibitors (claim 1);
6. 5,094,951 (Chiron) - glucose oxidase (claim 1);
7. 5,156,958 (American Cyanamid) - Bordetella pertussis membrane protein (claim 1);
8. 5,177,197 (Ludwig Institute) - human transforming growth factor B1 binding protein (claims 1 and 7);
9. 5,260,208 (Rhone-Poulenc Sante) - enantioselective amidases (claim 1); and
10. 5,416,013 (Sterling Winthrop) - interleukin 1B protease (claim 1).

The Examiner also contends that the specification "does not teach the concept of making DNA sequences of any size that generally contain regions of mismatch". In supposed support of this contention, the Examiner asserts that:

(a) "the specification does not describe generally comparing DR-β chain sequences to identify regions of mismatch and to then generate DNA sequences which contain those mismatches where the sequence can be of any length and are not limited to a particular disclosed DNA sequence";

(b) "the claims are drawn to DNA sequences which specifically hybridize to the polymorphic regions between DR-β-A and DR-β-B to allow determination of HLA alleles for use in DR-β [typing] which the specification does not describe" and "the specification is limited to describing the synthesis and use of these short 19-mer oligonucleotides that span the specific mismatch"; and

(c) "the specification only teaches that the region between amino acids 39-45 of the disclosed β-chain genes is conserved between the specific DR-β chain gene sequences described in the specification and teaches one 19-mer which spans this region for use as a hybridization control in the improved HLA typing method" and "does not describe that the invention includes any sequence which contains or is complementary to this conserved region because the specification teaches that this sequence is present in DC genes and SB-beta chains which are encompassed by the claims as

written but do not appear to be part of the intended invention". Applicants disagree.

Contrary to the Examiner's contention (a) above, the concept of aligning two DNA sequences, capable of hybridizing to applicants' disclosed sequences, in order to identify regions of mismatch, and to make DNA sequences of any length containing said mismatch regions, is fully supported by the disclosure of the instant application. As acknowledged by the Examiner, the specification teaches the comparison of DR-β-A and DR-β-B to identify three regions containing mismatches and one conserved region, and also teaches the production and use of short 19-mers which span the region of mismatch in order to distinguish perfectly matching sequences from mismatching sequences by hybridization as a means for HLA-DR typing (see pages 31-32). In view of such disclosure, it would have been obvious for one of skill of the art, at the June 30, 1982 effective filing date of the application, to employ applicants' illustrative processes to both compare additional DR-β chain sequences and to define and generate DNA sequences which contain additional mismatch sequences.

For example, Wallace et al., Nucleic Acid Res., Vol. 6, pp. 3543 (1979) [Exhibit C] and Wallace et al., Nucleic Acid Res., Vol. 9, pp. 879 (1980) [Exhibit D], determined that oligonucleotides hybridized to their complementary sequences with a high degree of specificity and that under appropriate conditions only duplexes will form in which all of the nucleotides are base paired, while mismatched duplexes will

not. As evidenced by those articles, it would have been obvious to one of skill in the art as of June 30, 1982 to design oligonucleotide probes that were a mixture of all possible coding sequences for a given amino acid sequence. Accordingly, given the teachings in the patent application, read in light of knowledge in the art as of its filing date, one of skill in the art would have know how to employ the disclosed HLA-β-A or HLA-β-B sequence and oligonucleotide probes in order to align DNA sequences, identify areas of mismatch and to make DNA sequences of varying lengths which contain said mismatch regions.

As opposed to the Examiner's contention (b) above, applicants believe that as of June 30, 1982, it would have been obvious to the skilled artisan that if a portion of the amino acid sequence of a protein was know (such as the HLA-β-A or HLA-β-B sequence disclosed by applicants), this information could be used to design an oligonucleotide probe for use in state of the art hybridization techniques to define allelic variants of HLA-DR-β. More particularly, several logical screening strategies had used synthetic oligonucleotides as probes for determination of hybridization. For example, oligonucleotides of unique sequences were know to be useful for screening recombinant DNA libraries (as discussed on pages 11-23 of the specification) and for the identification of cDNA clones by hybridization selection (see Sambrook et al., Molecular Cloning: A Laboratory Manual, pp. 329-352 (1982) [Exhibit E]). The hybridization specificity of oligonucleotide

probes made it possible to use unique sequence probes to screen for genomic clones or cDNAs encoding a specific member of a multigene family (see Schulze et al., Mol. Cell. Biol., Vol. 3, pp. 750 (1983) [Exhibit F]). Furthermore, Wallace et al., Science, Vol. 209, pp. 1396 (1980) [Exhibit G] and Wallace et al., Nucleic Acid Res., Vol. 9, pp. 3647 (1981) [Exhibit H] describe the use of unique nucleotide sequences to design probes to screen for a new allele when the sequence of one allele is know, to screen for a specific region of a gene, and to screen for specific mutants created by site-directed mutagenesis.

Furthermore, the concept of hybridization was well defined in the art, as "the pairing of two complementary or nearly complementary DNA strands to form a stable duplex". It was also known that the efficiency of such pairing is a function of both the temperature of the hybridization medium (which tends to dissociate the duplex) and the salt concentration of the medium (which tends to associate the duplex). Thus, at any given salt concentration, there is a particular temperature at which the two DNA strands of the hybrid duplex will dissociate from one another. This dissociation is termed "melting" and the temperature of melting is the Tm of the hybrid under the given salt conditions (see Bonner et al., J. Mol. Biol., Vol. 81, pp. 123 (1973) [Exhibit I]).

As a result of a large number of empirical observations, an equation has been developed that permits estimation of Tm at any given salt concentration. This equation is the Schildkraut relation:

$$Tm = 81.5 + 0.41(\%G+C) + 16.6(\log M) - 0.72(F\%)$$

wherein:

Tm = temperature of melting in Celsius degrees

(%G+C) = G/C content of DNA

M = salt (monovalent cation) concentration in moles/liter

F = formamide concentration (volume/volume).

Thus, for a particular salt concentration, the Tm of a hybrid duplex can be calculated. Theoretically, for effective hybridization, one could use any temperature below that estimated Tm to obtain the stable hybrid. However, in practice, the rate of hybridization is strongly dependent upon the particular temperature below Tm employed. In the definitive experiments of Bonner et al., the rate of hybridization was measured at various temperatures ranging from Tm-7 to Tm-50. These data are depicted in Figure 8 of the article. They show that the rate of hybridization reaches a peak between Tm-20 and Tm-27. From the breadth of the peak, it can be seen that hybridization temperatures of Tm-15 and Tm-30 give hybridization rates of over 90% of the maximum rate. Other experiments described in Bonner et al. have demonstrated that as the hybridization temperature decreases below Tm-27 the specificity of the hybridization progressively worsens. Thus,

as a result of Bonner's experiments, an optimal range of hybridization conditions has been defined to be from about Tm-20 to Tm-27. It would be recognized by one of skill in the art at the effective filing date of this application that, for the disclosed purpose, salt and temperature conditions that result in this range of Tm would be an example of known hybridization conditions able to effect correct base pairing between nucleotide strands.

Furthermore, the classic formula to calculate the effect of mismatches on the stability of long DNA hybrids put forth by Bonner et al. in 1973, may be applied for hybrids involving short nucleotides (as confirmed by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, pp. 11.47 (1989) [Exhibit J]). With applicants' disclosure in hand, and in view of knowledge in the art as of the effective filing date of this application, it is clear that a skilled artisan could readily prepare DNA sequences, other than those of specifically "defined sequence composition", and employ said sequences for HLA-DR typing processes and kits according to the present invention.

Finally, with respect the Examiner's contention (c) above, applicants wish to clarify that the "conserved" region of HLA-DR-β chain genes (the coding sequence for amino acids 39-45) would serve the function of a "positive" control for confirmation that the an particular set of hybridization conditions is suitable for achieving accurate hybridization. Contrary to the Examiner's contention, that region would not

have a "direct" function in the selection of HLA-DR-β alleles, other than that of providing an initial source of HLA-β chain sequences for further DR-β chain screening. See added claims 70-72.

With respect to the prior § 112, first paragraph rejection (see pages 6-7 of the Office Action), the Examiner maintains that applicants' arguments "have been thoroughly reviewed but are deemed non-persuasive because they are allegations which are not supported by the level of routine experimentation at the time of filing". Applicants traverse.

As demonstrated in the foregoing discussion, Bonner et al. (1976), Wallace et al. (1979), Wallace et al. (1980), Wallace et al. (1981), Sambrook et al. (1982), Wallace et al. (1983) and Schulze et al. (1983) fully describe how synthetic oligonucleotides and the technology of hybridization could be combined to: (1) design an oligonucleotide probe that is a mixture of all possible coding sequences for a given amino acid sequence, (2) screen recombinant DNA libraries, (3) identify cDNA clones by hybridization selection, (4) use unique sequence probes to screen for genomic clones or cDNAs encoding a specific member of a multigene family, (5) use unique nucleotide sequences to design probes to screen for a new allele when the sequence of one allele is know, (6) screen for a specific region of a gene and (7) screen for specific mutants created by site-directed mutagenesis. Applicants reiterate that, at their filing date, one of skill in the art would appreciate that, in any specific DNA typing kit or typing

process, the useful DNA sequences are those that selectively hybridize to the DNA sequence of interest, and that it would also be routine for one of skill in the art to determine the length of a DNA sequence that will hybridize to a particular DNA sequence in the human genome. Thus, once provided with a particular DNA sequence, such as those of "defined sequence composition" illustrated in the application and recited in the claims, one of skill in the art would readily be able to prepare other DNA sequences or fragments of those DNA sequences that would hybridize to DNA of interest.

Applicants now address the Examiner's new grounds of rejection set forth on pages 8-11 of the Office Action.

The Examiner objects to Figures 5-5D and 7-7A and the brief descriptions for these figures on the ground they should instead be designated as Figures 5A-5E and 7A-7B, respectively. Applicants have amended the specification to designate Figures 5A-5E and 7A-7B accordingly. Further, upon notification of allowable claims in this application, applicants stand ready to file formal drawings which reflect the above-mentioned amendments.

The Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 16, 17, 20, 23-42, 44, 46, 47, 49 and 50 stand rejected under 35 U.S.C. § 112, first paragraph, on the basis that "the claims are not enabled by the specification because the claims encompass DNA sequences of DR-β-C and DR β-D which have not been taught in the specification". The Examiner

states that "a clone containing DR-β-C was deposited with the ATCC but the deposit has not been perfected" and "a deposit of clones containing this sequence according to the requirements set forth in C.F.R. §§ 1.801-1.809 and amendment of the specification to the required statements regarding the deposit according to the rules is required".

Applicants have canceled claims which encompass the DR-β-D sequence, thereby obviating the rejection with respect thereto. Furthermore, the clone containing the sequence of DR-β-C has been deposited with the Patent Culture Depository of the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Virginia 20110-2209, U.S.A., under ATCC Accession Number 39165. That deposit was made in conjunction with United States patent 5,503,976, which issued on April 2, 1996. Applicants attach hereto as Exhibit K, a copy of the Declaration of Margaret A. Pierri, attorney for applicants, regarding the clones containing the sequences of HLA-DR-β-A, HLA-DR-β-B and HLA-DR-β-C (ATCC Numbers 39164, 39163 and 39165, respectively). Exhibit 1 to that Declaration is a copy of the ATCC Deposit Receipt.

As-indicated on the Deposit Receipt, the material deposited under ATCC Numbers 39164, 39163 and 39165 will be maintained for a period of 30 years from the date of the deposit (i.e. July 28, 2012).

In view of the foregoing amendments and remarks, applicants believe that the Examiner's rejections based on asserted new matter and non-enablement are moot. This is all

the more so, in view of the fact that the Examiner has provided no evidence for the assertions of non-enablement. In the absence of such evidence, the Examiner has no reason to doubt the objective truth of applicants' asserted operability of the present invention:

> "As a matter of Patent Office practice ... a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented, must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied upon for enabling support... [I]t is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." Ex parte Kenaga, 189 USPQ 62, 64 (Pat. Off. Bd. Pat. App. 1975), quoting In re Marzocchi, 439 F.2d 220, 223-24, 169 USPQ 367, 369 (CCPA 1971) (emphasis in original).

The Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 16, 17, 20, 23-42, 44, 46, 47, 49 and 50 stand rejected under 35 U.S.C. § 112, second paragraph, as being "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention", based on terminology objected to by the Examiner. More particularly, the Examiner states that:

(a) claims 16, 17, 20, 23, 24, 26-30, 34-37, 44, 46 and 49 and are indefinite because of the current "Markush" format;

(b) claims 16, 17, 20, 23, 24, 26-30, 34-36, 46 and 49 are indefinite over the recitation in part (e) of "DNA sequences

which differ...., because the claims are unclear as to whether "said DNA sequence" is to contain multiples of the previously recited sequences or only one";

(c) claims 31-33, 36, 37, 40-42, 44, 47 and 50 are indefinite over the recitation of "said polymorphic regions being encoded by DNA selected from the group consisting of: (a) DNA sequences encoding....., because the claims are unclear as to whether the DNA of the polymorphic regions is multiple sequences encoding the recited amino acid regions or a single DNA sequence"; and

(d) claims 34-37 are indefinite over the recitation of "said DNA inserts" because this term lacks antecedent basis.

Applicants have obviated each of the Examiners' contentions set forth in (a)-(d) by virtue of the cancellation of claims 16, 17, 20, 23-42, 44, 46, 47, 49 and 50. Furthermore, applicants have added claims 51-72, which incorporate all of the changes to claim language suggested by the Examiner.

The Rejection Under 35 U.S.C. § 102(b)

Claims 23, 24, 26-29, 31-33, 36 and 37 stand rejected under 35 U.S.C. § 102(b) as being "anticipated by" Larhammar et al. (Proc. Nat. Acad. Sci., USA, June 1992, 79: 3687-3691). More specifically, the Examiner asserts that "Larhammar teach a DNA sequence encoding an amino acid sequence of an HLA-β chain locus". Referring to Larhammar et al., the Examiner acknowledges that "the nucleotide and predicted amino acid

sequence are different from the disclosed DR-β-A and DR-β-B sequence. However, the Examiner contends that because the intended meaning of the term "specifically hybridize" is unclear as discussed above, this DNA would be encompassed by the claims as written". This rejection is obviated by applicants' cancellation of claims 23, 24, 26-29, 31-33, 36 and 37. Added claims 51-72 are directed to HLA-DR typing processes and kits employing, for example, the HLA-DR-β-A and HLA-DR-β-B sequences and the regions of mismatch between these two sequences. Applicants believe that the DC-β chain sequence described by Larhammar et al. does not anticipate or render obvious the use of the HLA-β-A and HLA-β-B sequences and the regions of mismatch between these two sequences, to design the HLA typing processes and kits according to the present application.

The Double Patenting Rejections

Claims 16, 17, 20, 22-42, 44, 46, 47, 49 and 50 stand rejected under the doctrine of obviousness-type double patenting as being "unpatentable over" claims 1-22 of United States patent 5,169,941. Applicants have obviated this rejection by canceling those claims. Furthermore, applicants believe that this rejection is moot, by virtue of applicants' amendment of this application to divisional status. More particularly, this application is now a divisional of United States Serial No. 07/902,999 (now United States patent 5,503,976), which is in turn a divisional of United States

Serial No. 06/518,393 (now United States patent 5,169,941). The claims now pending relate to HLA-DR typing kits and processes. Such kits and processes were divided from both the parent applications which issued as the foregoing patents as a result of restriction requirements. Accordingly, pursuant to MPEP § 804.1, no terminal disclaimer should be required.

In addition, claims 16, 17, 20, 30, 40, 41, 42, 44, 46, 47, 49 and 50 stand rejected under the doctrine of obviousness-type double patenting as being "unpatentable over" claims 1-10 of United States patent 5,503,976. Should the Examiner maintain the double patenting rejection in view of the claim amendments, applicants stand ready to file a Terminal Disclaimer in this application, if appropriate.

Applicants acknowledge, with appreciation, the Examiner's withdrawal of the previous rejection of claims 23-24 and 31 under 35 U.S.C. § 112, second paragraph, in view of the amendments thereto.

Applicants request that the Examiner consider the foregoing amendments and remarks and pass this application to issue.

Respectfully submitted,

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